USER GUIDE

invitrogen™
by life technologies™

DES® Blasticidin Support Kit

Catalog number K5150-01

Revision date 20 February 2012
Publication Part number 25-0397

MAN0000211

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

life technologies™

Contents

Kit Contents and Storage

Shipping and Storage

Shipping:

- The Schneider (S2) Cells are shipped on dry ice.
- The rest of the kit is shipped at room temperature.

Storage: Upon receipt,

- Store the cells in liquid nitrogen
- Store the blasticidin, pCoBlast vector, and the Calcium Phosphate Transfection Kit at –30°C to –10°C
- Store Schneider's *Drosophila* Medium at 2°C to 8°C

Kit Contents

The DES® Blasticidin Support Kit contains the following reagents. Store as indicated in the following table.

Item	Amount Supplied	Storage
pCoBlast vector	40 µL at 0.5 µg/µL in TE, pH 8	–30°C to –10°C
Blasticidin	50 mg, powder	–30°C to –10°C
Schneider (S2) Cells	1 vial containing 1 mL of cells at 1×10^7 cells/mL in Freezing Medium	Liquid nitrogen
Schneider's *Drosophila* Medium	2 × 500 mL	2°C to 8°C

Calcium Phosphate Transfection Kit

The Calcium Phosphate Transfection Kit included with each DES® Blasticidin Support Kit contains the following reagents. Sufficient reagents are provided for 75 transfections. Store at –30°C to –10°C.

Note: The Calcium Phosphate Transfection Kit is also used to transfect mammalian cells. The positive control vector pcDNA™3.1/His/*lac*Z is included for mammalian cell transfection. Do not use this vector as a control for transfection into S2 cells. Use an appropriate *Drosophila* expression vector (e.g. one of the control expression plasmids included in the DES® Kits available from Life Technologies).

Item	Concentration	Amount Supplied
Tissue Culture Sterile Water	—	2 × 12 mL
2X HEPES Buffered Saline (HBS)	50 mM HEPES 1.5 mM Na_2HPO_4 280 mM NaCl pH 7.1	2 × 12 mL
$CaCl_2$	2 M	3 × 1 mL
pcDNA™3.1/His/*lac*Z	40 µL at 0.5 µg/µL in TE, pH 8	20 µg (for mammalian transfection only)

Product use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Introduction

Product Overview

Description of the System

The DES® Blasticidin Support Kit contains reagents specifically designed for use with the *Drosophila* Expression System (DES®) available from Life Technologies. When used in conjunction with an appropriate expression vector, the DES® Blasticidin Support Kit contains the pCoBlast selection vector and other reagents necessary to generate stable cell lines expressing your gene of interest in *Drosophila* Schneider 2 (S2) cells. Stable cell lines expressing heterologous proteins can be generated in 2 weeks following cotransfection of the expression vector and pCoBlast. By optimizing the ratio of expression vector to selection vector, you may generate cell lines with a very high copy number of the desired gene, leading to increased expression levels of the desired protein.

pCoBlast

pCoBlast is a 3.9-kb selection vector which, when cotransfected with a DES® expression vector, allows you to generate stable S2 cell lines expressing the heterologous gene of interest. The vector contains the following elements:

- The *Drosophila copia* promoter for high-level, constitutive expression of the blasticidin resistance gene
- The blasticidin resistance gene (*bsd*) for selection of stable *Drosophila* cell lines (Kimura *et al.*, 1994)
- Ampicillin resistance gene for selection of the plasmid in *E. coli*

Expression Vectors

The expression vectors used in the DES® System are versatile, allowing inducible expression from the metallothionein (MT) promoter (Bunch *et al.*, 1988; Maroni *et al.*, 1986) or constitutive expression from the Ac5 promoter (Chung and Keller, 1990). Expression can either be intracellular or secreted for simplified purification. The following table lists the expression vectors available from Life Technologies and their function. Many native signal sequences are functional in S2 cells and can be used to secrete proteins using either the pAc5.1/V5-His or pMT/V5-His vectors. pMT/BiP/V5-His is also available if you want to fuse your protein to a *Drosophila* secretion signal sequence.

The expression vectors are available separately or as part of a DES® System Kit (see page 21). For more information about any of the *Drosophila* expression vectors, refer to the manual for each vector. The manuals are available from **www.lifetechnologies.com/manuals** or by contacting Technical Support (see page 22).

Expression Vector	Function	Cat. no.
pAc5.1/V5-His A, B, and C	Constitutive expression of heterologous proteins	V4110-20
pMT/V5-His A, B, and C	Inducible expression of heterologous proteins	V4120-20
pMT/BiP/V5-His A, B, and C	Inducible, secreted expression of heterologous proteins	V4130-20
pMT/BioEase™-DEST Gateway® Vector	Inducible expression of biotinylated, heterologous proteins	V4140-20

Continued on next page

Generating Stable S2 Cell Lines

The following table outlines the basic steps necessary to generate a stable S2 cell line using pCoBlast and a suitable expression plasmid. For more details, refer to the pages indicated.

Step	Action	Page
1	Establish culture of S2 cells from supplied frozen stock.	3–6
2	Clone your gene of interest into a suitable *Drosophila* expression vector. Prepare purified DNA from the recombinant expression vector.	see vector manual
3	Prepare purified pCoBlast plasmid DNA.	9
4	Cotransfect the recombinant expression vector with the selection vector, pCoBlast, and select for transformants using blasticidin.	9–12
5	Induce, if necessary, and assay for expression of your protein.	12

Methods

Culturing S2 Cells

Introduction

A vial of frozen *Drosophila* Schneider 2 (S2) cells is included in the DES® Blasticidin Support Kit. The S2 cell line was derived from a primary culture of late stage (20–24 hours old) *Drosophila melanogaster* embryos (Schneider, 1972). Many characteristics of the S2 cell line suggest that it is derived from a macrophage-like lineage. S2 cells grow at 28°C without CO_2 as a loose, semi-adherent monolayer in tissue culture flasks and in suspension in spinners and shake flasks.

General Cell Handling

General guidelines are provided below to help you grow S2 cells.

- All solutions and equipment that come in contact with the cells must be sterile.
- Always use proper sterile technique in a laminar flow hood.
- All incubations are performed in a 28°C incubator and do not require CO_2.

  Note: If you want to slow down S2 cell growth, you may incubate cells at room temperature (22–25°C).
- The complete medium for S2 cells is Schneider's *Drosophila* Medium containing 10% **heat-inactivated** fetal bovine serum (FBS). This medium can be used for transient expression and stable selection. Schneider's *Drosophila* Medium and FBS are available separately (see page 21 for ordering information).
- Adding antibiotics is not recommended; however, 5 mL/L of Penicillin-Streptomycin (see page 21 for ordering information) may be used when required.
- Before starting experiments, be sure to have established frozen S2 cell stocks.
- Count the cells before seeding for transfection or freezing cells for stocks. Check for viability (if desired) using trypan blue. S2 cell viability in culture should be >95%.
- Always use **new** flasks or plates when passing cells for general maintenance. During transfection and selection keep cells in the **same** culture vessel.
- S2 cells grow better if some conditioned medium is brought along when passaging cells.

  Note: Conditioned medium is medium in which cells have been grown.

Important

S2 cells do not completely adhere to surfaces, making it difficult to rinse the cells if needed. To exchange cells into new medium or to wash cells prior to lysis:

- Resuspend cells in the conditioned medium and centrifuge at 100 × g for 5–10 minutes. Decant the medium.
- Resuspend the cells in fresh medium (or PBS) and centrifuge again at 100 × g for 5–10 minutes. Repeat.
- Add fresh medium (or buffer) and replate the cells (or lyse them).

Continued on next page

Materials Required but not Supplied

- 15-mL sterile, conical tubes
- 5-, 10-, and 25-mL sterile pipettes
- Cryovials
- Reagents to count cells
- Heat-inactivated fetal bovine serum (FBS)
- Penicillin-Streptomycin, if desired (see page 21 for ordering information)
- Table-top centrifuge
- 25-cm^2 flasks, 75-cm^2 flasks, and 35-mm plates (other flasks and plates may be used)
- Phosphate-Buffered Saline (see page 21 for ordering information)
- Complete Schneider's *Drosophila* Medium: Schneider's *Drosophila* Medium (included with the kit) containing 10% **heat-inactivated** fetal bovine serum (FBS)

Thawing Cells

Store frozen cells in liquid nitrogen until ready to use. Use the following procedure to thaw cells.

1. Remove the vial of cells from liquid nitrogen and thaw quickly in a 37°C water bath. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol.
2. Triturate and transfer the entire contents of the cryovial into a sterile, conical tube containing 4 mL of room temperature, complete Schneider's *Drosophila* Medium.
3. Centrifuge at 100 × g for 5–10 minutes. Aseptically decant the medium containing DMSO and resuspend the cell pellet in 5 mL of fresh complete Schneider's *Drosophila* Medium.
4. Transfer the 5 mL cell suspension into a 25-cm^2 flask, and incubate in a 28°C non-humidified, ambient air-regulated incubator or warm room. Loosen caps of flasks to allow oxygenation/aeration.
5. After the culture density has reached $>2 \times 10^6$ viable cells/mL, subculture cells at a seeding density of 5×10^5 viable cells/mL in complete growth medium.
6. After the culture density has reached $>1 \times 10^7$ viable cells/mL, subculture cells at a seeding density of 5×10^5 viable cells/mL in complete growth medium. See **Subculturing Cells**, next page, to main and subculture S2 cells.

Important

For the first day after thawing, many of the S2 cells may be floating in the medium and will look smaller in size. The majority of these cells are viable although some may take up trypan blue stain. During this time, do not use trypan blue staining to count the cells. Because some viable cells can take up trypan blue, your cell counts may be inaccurate. After the cells have recovered from the thawing procedure, use of trypan blue staining to count cells is acceptable.

Continued on next page

Subculturing Cells

For general maintenance of cells, pass S2 cells when the culture density has reached 6 to 20 × 10^6 viable cells/mL. Use the following procedure to subculture cells.

Note: Cells will start to clump at a density of ~5 × 10^6 cells/mL in serum-containing medium. This does not seem to affect growth. Clumps can be broken up during passage.

1. To remove cells from the flask, tap the flask several times to dislodge cells that may be attached to the surface of the flask. Use a 5 mL pipette to wash down the surface of the flask with the conditioned medium to remove the remaining adherent S2 cells. Transfer the cell suspension into a sterile, conical tube.
2. Determine the viability and total cell counts.
3. Seed cells at a density of 5 × 10^5 viable cells/mL. Do not seed cells below a density of 5 × 10^5 cells/mL.

   Tip: Dilute cells in a total working volume of 12–15 mL fresh, complete medium for a 75-cm^2 flask and 40–50 mL for a 162-cm^2 flask.
4. Put flasks in a 28°C non-humidified, ambient air-regulated incubator or warm room. Loosen caps of flasks to allow oxygenation/aeration.
5. When the culture density reaches 6 to 20 × 10^6 viable cells/mL, repeat steps 1–4 as necessary to expand cells for transfection or expression.

Continued on next page

Freezing S2 Cells

Recommended Conditions

- Freeze cells at a density of ≥1 × 10^7 viable cells/mL.
- Use a freezing medium composed of 50% fresh, complete growth medium and 50% conditioned growth medium (day 2 to 4 cell conditioned medium collected from S2 cultures during subculturing procedure) and DMSO to a final concentration of 10%. Prepare freezing medium immediately before use. Filter-sterilize the freezing medium and chill at 4°C until use. Discard any remaining freezing medium after use.

  Important: Optimal recovery of S2 cells requires growth factors in the medium. Be sure to include **conditioned** medium in the Freezing Medium. In addition, FBS that has not been heat-inactivated will inhibit growth of S2 cells.

Procedure

1. Grow the desired quantity of S2 cells in shake or spinner flasks, harvesting when the cells are in mid-log exponential growth and have a viability of >90%.
2. Determine viability and total cell counts and calculate the volume of freezing medium required to yield a final cell density of ≥1 × 10^7 viable cells/mL.
3. Prepare the required volume of freezing medium (see **Recommended Conditions**).
4. Centrifuge cells from cell suspension (step 1 of this procedure) at 100 × g for 5–10 minutes. Aseptically decant the supernatant and resuspend the cell pellet in the pre-determined volume of chilled freezing medium**.**
5. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer's specifications (i.e. 1 mL in a 1.5-mL cryovial).
6. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
7. Transfer frozen vials to liquid nitrogen storage.

   Note: You may check the viability and recovery of frozen cells 24 hours after storing vials in liquid nitrogen by following the procedure outlined in **Thawing Cells**, page 4.

Propagate and Maintain pCoBlast

E. coli Host

Many *E. coli* strains are suitable for propagating pCoBlast vectors including TOP10 or DH5α™-T1^R. We recommend that you propagate pCoBlast vectors in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10 and DH5α™-T1^R *E. coli* are available as chemically competent or electrocompetent (TOP10 only) cells in a One Shot® format.

Item	Quantity	Cat. no.
One Shot® TOP10 (chemically competent cells)	21 × 50 µL	C4040-03
One Shot® TOP10 Electrocomp (electrocompetent cells)	21 × 50 µL	C4040-52
One Shot® DH5α™-T1^R Max Efficiency® (chemically competent cells)	21 × 50 µL	12297-016

Transformation Method

You may use any method of choice for transformation. Chemical transformation is the most convenient for many researchers. Electroporation is the most efficient and the method of choice for large plasmids.

Plasmid Maintenance

The pCoBlast vector contains the ampicillin gene to allow selection of the plasmid using ampicillin (see page 19 for more information about the vector).

To propagate and maintain the plasmid, we recommend using the following procedure:

1. Transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α™-T1^R, or equivalent.
2. Select transformants on LB agar plates containing 50–100 µg/mL ampicillin. To prepare Low Salt LB agar containing ampicillin quickly and easily with a microwave, use imMedia™ Amp Agar (see page 21 for ordering information).
3. Prepare a glycerol stock of each plasmid for long-term storage (see the next page).

Continued on next page

Prepare a Glycerol Stock

After identifying the correct clone, purify the colony and make a glycerol stock for long-term storage. We also recommend keeping a DNA stock of your plasmid at –20°C.

1. Streak the original colony out on an LB plate containing 50–100 g/mL ampicillin. Incubate the plate at 37°C overnight.
2. Isolate a single colony and inoculate into 1–2 mL of LB containing 50–100 g/mL ampicillin.
3. Grow the culture to mid-log phase (OD_{600} = 0.5–0.7).
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
5. Store at −80°C.

Generating Stable Cell Lines

Introduction

To generate stable S2 cell lines, you will cotransfect the pCoBlast selection vector and the recombinant expression vector into S2 cells and select for stable transfectants using blasticidin. Calcium phosphate precipitation is generally the method of choice to transfect S2 cells.

Drosophila stable cell lines generally contain multicopy inserts that form arrays of more than 500–1000 copies in a head to tail fashion (Kirkpatrick and Shatzman, 1997). Manipulate the number of inserted gene copies by varying the ratio of expression and selection plasmids. We recommend using a 19:1 (w/w) ratio of expression vector to selection vector. You may vary the ratios to optimize expression of your particular gene. We also recommend that you include a positive control and a negative control in your transfection experiment to help you evaluate your results.

Generating the Expression Construct

If you are using one of the DES® expression vectors, a separate manual is provided to facilitate cloning your gene of interest into the DES® expression vector. For maps and diagrams of the multiple cloning site, refer to the specific manual for each vector. The sequence of each DES® expression vector is available from **www.lifetechnologies.com** or by calling Technical Support (see page 22). After obtaining your expression construct, prepare purified plasmid DNA for transfection into S2 cells.

Plasmid Preparation

Plasmid DNA for transfection into S2 cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink® HiPure Filter Plasmid Kit (see page 21 for ordering information) or CsCl gradient centrifugation. The PureLink® HiPure Filter Plasmid Kit is a medium-scale plasmid isolation kit that isolates up to 200 µg of plasmid DNA from 25–100 mL of bacterial culture. Plasmid can be used directly for transfection of S2 cells.

pCoBlast and Blasticidin Selection

The pCoBlast selection vector expresses the blasticidin resistance gene (*bsd*) from the *Drosophila copia* promoter (see page 17 for a map of the plasmid) and allows selection of stable transfectants using blasticidin. Blasticidin is supplied with the DES® Blasticidin Support Kit and is also available separately (see page 21 for ordering information). For instructions to prepare and handle blasticidin, see the **Appendix**, page 15.

Continued on next page

Note

- We recommend using Schneider's *Drosophila* Medium to select stable S2 cell lines with blasticidin. After stable cell lines have been generated, you may maintain cells in Schneider's *Drosophila* Medium containing the appropriate concentration of antibiotic.
- It may be possible to use serum-free medium to select stable S2 cell lines; however, note that some serum-free media can **only** be used for recombinant protein expression and purification purposes and **cannot** be used for blasticidin selection. Addition of blasticidin to some serum-free media will kill S2 cells, even those that are blasticidin-resistant in serum-containing medium. If you want to use serum-free medium for selection, we suggest that you test your serum-free medium directly.

Blasticidin Selection Guidelines

To select for S2 cells that have been stably cotransfected with pCoBlast and a DES® expression vector, we generally use 25 µg/mL of blasticidin. If this concentration does not work for you, we recommend that you perform a kill curve:

- Prepare complete Schneider's *Drosophila* Medium supplemented with a range of blasticidin concentrations (e.g. 5–100 µg/mL blasticidin).
- Test varying concentrations of blasticidin on the S2 cell line to determine the concentration that kills your cells (kill curve).

Selection with blasticidin is generally much faster than selection with other antibiotics. We typically observe complete inhibition of cell growth after 2 weeks in selective medium. Cell death may be verified using trypan blue staining.

Required Materials

Components required but not supplied:

- S2 cells growing in culture
- 35-mm plates (other flasks or plates can be used)
- Complete Schneider's *Drosophila* Medium (see page 15)
- Recombinant DNA (19 µg per transfection)
- Sterile microcentrifuge tubes (1.5-mL)
- Cell Lysis Buffer (see recipe on page 15)

Components supplied with the kit:

- pCoBlast (0.5 µg/µL solution in TE; use 1 µg per transfection)
- Blasticidin
- Calcium Phosphate Transfection Kit

Continued on next page

Transfection Procedure

Follow the steps below to stably transfect cells. Use a 19:1 ratio of expression vector to selection vector. Set up several transfections with different ratios of expression vector to selection vector to optimize expression of your protein. Include appropriate controls.

Day 1: Prepare

1. Seed 3×10^6 S2 cells in a 35-mm plate in 3 mL complete Schneider's *Drosophila* Medium.
2. Grow the cells for 6–16 hours at 28°C until the cells reach log phase ($2–4 \times 10^6$ cells/mL).

Day 2: Transfect

3. Prepare the following transfection mix for a 35-mm plate:

   In a microcentrifuge tube, mix together the following components. This will be **Solution A**.

2 M $CaCl_2$	36 µL
Recombinant DNA (19 µg)	*x* µL
pCoBlast (1 µg)	2 µL
Tissue culture sterile water	Bring to a final volume of 300 µL

   To a second microcentrifuge tube, add 300 µL 2X HBS. This is **Solution B**.
4. Slowly add **Solution A** dropwise to **Solution B** with continuous mixing. Continue adding and mixing until **Solution A** is depleted.
5. Incubate the resulting solution at room temperature for 30–40 minutes. After ~30 minutes, a fine precipitate will form.
6. Mix the solution and add dropwise to the cells. Swirl the plate to mix in each drop after it is added. Incubate for 16–24 hours at 28°C.

Day 3: Posttransfection

7. Remove the calcium phosphate solution. Wash the cells twice with complete medium. To wash cells, resuspend cells in complete medium and centrifuge at 100 × g for 5–10 minutes. Decant the medium. Add fresh complete growth medium **(no selection agent)** and replate into the same well or plate. Do not split cells.
8. Incubate at 28°C for 2 days.

Day 5: Select

9. Centrifuge the cells and resuspend in complete growth medium containing 25 µg/mL blasticidin. Replace selective medium every 4–5 days until resistant cells appear (2 weeks). Always replate into old plates.

+2 Weeks: Expansion

10. Replate resistant cells into new plates with medium containing blasticidin and pass cells when they reach a density of 6 to 20×10^6 cells/mL. This is to remove dead cells. **Note:** You may plate resistant cells into smaller plates or wells to promote cell growth before expansion for large-scale expression or for preparing frozen stocks.

Continued on next page

Transfection Procedure, Continued

11. Expand resistant cells into 6-well plates to test for expression or into flasks to prepare frozen stocks. Maintain cells in medium containing blasticidin.

    Note: After obtaining stable S2 cell lines, you may maintain the cells in medium containing lower concentrations of blasticidin. We typically maintain stable S2 cell lines in Schneider's *Drosophila* Medium containing 10 µg/mL blasticidin.

Assay for Expression

Induce, if necessary, and assay each of your stable cell lines for yield of the desired protein by western blot or functional assay. Select the cell line with the highest recombinant protein yield for scale-up and purification of recombinant protein. **If your protein is secreted, remember to assay the medium.** You may wish to compare the yield of protein in the cells and supernatant.

If you intend to assay for expression of your recombinant protein using western blot analysis, use the following protocol.

Test for Expression

Use the cells from one 35-mm plate for each expression experiment.

1. Prepare an SDS-PAGE gel that will resolve your expected recombinant protein.
2. Transfer cells to a sterile, 1.5-mL microcentrifuge tube. **If your recombinant protein is secreted, be sure to save and assay the medium.**
3. Pellet cells at 100 × g for 5–10 minutes. Transfer the supernatant (medium) to a new tube and resuspend the cells in 1 mL PBS.
4. Pellet cells and resuspend in 50 µL Cell Lysis Buffer (see page 15 for a recipe). Other recipes are suitable. Vortex.
5. Incubate the cell suspension at 37°C for 10 minutes. **Note:** You may prefer to lyse the cells at room temperature or on ice if degradation of your protein is a potential problem.
6. Vortex and pellet nuclei and cell debris. Transfer the supernatant to a new tube. Assay the lysate for protein concentration.
7. Add 4X SDS-PAGE sample buffer (see page 15 for a recipe) to a final concentration of 1X and boil the sample for 5 minutes.
8. Load approximately 3–30 µg of lysate onto an SDS-PAGE gel. The amount loaded depends on the amount of your protein produced. Load varying amounts of lysates or medium.
9. Electrophorese your samples; blot; and probe with a suitable antibody.
10. Visualize proteins using your method of choice.

Continued on next page

Scale-Up and Purification

After obtaining stable cell lines expressing the protein of interest and preparing frozen stocks of your cell lines, you are ready to scale-up expression and purify your recombinant protein. The strategy that you will use to purify your recombinant protein will vary depending on the nature of your protein. Refer to published reference sources for assistance (Coligan *et al.*, 1998; Deutscher, 1990). If you are using one of the DES® expression vectors to express your recombinant protein, refer to the DES® System manual for general guidelines for scale-up and purification.

Scaling-Up Cell Culture

To scale up the S2 cell culture, refer to the table on page 17 for the recommended volumes to use in various culture vessels. There is a protocol on page 18 for growing cells in suspension culture in either spinners or shake flasks.

Troubleshooting

Observation	Reason	Solution
Cells Growing Too Slowly (Or Not At All)	Cells were seeded at too low of a density during subculturing	Do not seed cells at less than 5×10^5 viable cells/mL. Cells will eventually grow back up if they were not seeded at too low of a density. If cells do not seem to be growing, thaw a new vial of cells.
	Conditioned medium is not used throughout passaging	Cells grow better if conditioned medium is used throughout passaging.
Low Transfection Efficiency	Impure plasmid DNA	Use pure plasmid DNA isolated using the PureLink® HiPure Filter Plasmid Kit or CsCl gradient ultracentrifugation.
	Improper transfections technique	• Make sure the calcium phosphate precipitate is fine enough. Be sure to thoroughly and continuously mix Solution B while you are adding Solution A. • S2 cells may also be transfected using some lipid-based transfection reagents including Cellfectin® II Reagent (see page 21 for ordering information) and dimethyldioctadecylammonium bromide (DDAB) (Han, 1996). For more information about Cellfectin® II Reagent and lipid-mediated transfection, contact Technical Support (page 22).
Low or No Protein Expression	Gene not cloned in-frame with signal sequence or appropriate N- or C-terminal tag	If your protein is not in frame with the BiP signal sequence, it will not be expressed or secreted. If it is not in frame with the appropriate N- or C-terminal tag, expression will not be detected using the appropriate reagent (e.g. V5 antibody or streptavidin conjugate).
	No Kozak sequence for proper initiation of transcription	Without a Kozak sequence, translation will be inefficient and the protein will not be expressed at its optimal level.
	Gene product is toxic to S2 cells	Use one of the inducible DES® vectors (i.e. pMT/V5-His, pMT/BiP/V5-His, or pMT/BioEase™-DEST).

Appendix

Recipes

Complete Schneider's *Drosophila* Medium

Schneider's *Drosophila* Medium is included in the DES® Blasticidin Support kit. It contains 2 mM L-glutamine. To prepare complete Schneider's *Drosophila* Medium, you need to add heat-inactivated fetal bovine serum (FBS) to a final concentration of 10% before use with *Drosophila* S2 cells.

FBS can be purchased already heat-inactivated or you may heat it at 65°C for 30 minutes to inactivate complement.

Optional: 5 mL/L of Penicillin-Streptomycin (see page 21 for ordering information) may be added to the medium, if desired.

Cell Lysis Buffer

50 mM Tris, pH 7.8
150 mM NaCl
1% Nonidet P-40

1. Prepare Cell Lysis Buffer from the following common stock solutions. For 100 mL, combine

1 M Tris base	5 mL
5 M NaCl	3 mL
Nonidet P-40	1 mL

2. Bring the volume up to 90 mL with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume up to 100 mL. Store at room temperature.

Note: Just before use, you may add protease inhibitors to a small volume of lysis buffer at the following final concentrations:

1 mM PMSF
1 µg/mL pepstatin
1 µg/mL leupeptin

4X SDS-PAGE Sample Buffer

Combine the following reagents:

0.5 M Tris-HCl, pH 6.8	5 mL
Glycerol (100%)	4 mL
β-mercaptoethanol	0.8 mL
Bromophenol Blue	0.04 g
SDS	0.8 g

Yield is ~10 mL.

Aliquot and freeze at −20°C until needed.

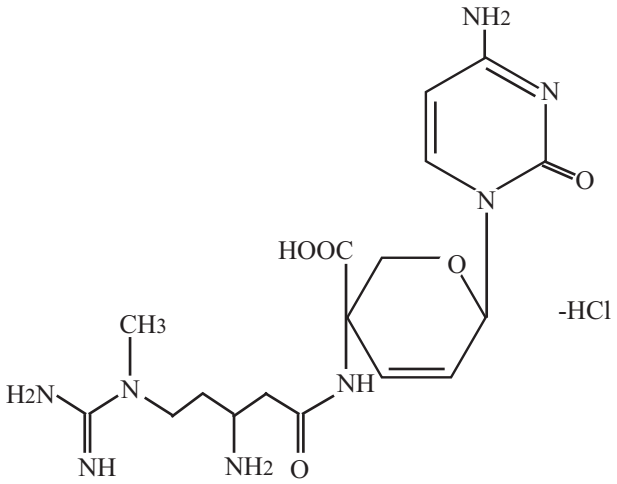
Blasticidin

Blasticidin

Blasticidin S HCl is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes* which inhibits protein synthesis in both prokaryotic and eukaryotic cells (Takeuchi *et al.*, 1958; Yamaguchi *et al.*, 1965). Resistance is conferred by expression of either one of two blasticidin S deaminase genes: *bsd from Aspergillus terreus* (Kimura *et al.*, 1994) or *bsr* from *Bacillus cereus* (Izumi *et al.*, 1991). These deaminases convert blasticidin S to a non-toxic deaminohydroxy derivative (Izumi *et al.*, 1991).

Molecular Weight, Formula, and Structure

The formula for blasticidin S is $C_{17}H_{26}N_8O_5$-HCl, and the molecular weight is 458.9. The diagram below shows the structure of blasticidin.



Handling Blasticidin

Always wear gloves, mask, goggles, and protective clothing (e.g. a laboratory coat) when handling blasticidin. Weigh blasticidin and prepare solutions in a hood.

Preparing and Storing Stock Solutions

Blasticidin is supplied in the DES® Blasticidin Support Kit, but may also be obtained separately (see page 21 for ordering information) in 50 mg aliquots. Blasticidin is soluble in water. Sterile water is generally used to prepare stock solutions of 5–10 mg/mL.

- Dissolve blasticidin in sterile water and filter-sterilize the solution.
- Aliquot in small volumes suitable for one time use and freeze at –20°C for long-term storage or store at 4°C for short-term storage.
- Aqueous stock solutions are stable for 1–2 weeks at 4°C and 6–8 weeks at –20°C.
- The pH of the aqueous solution should be 7.0 to prevent inactivation of blasticidin.
- Do not subject stock solutions to freeze/thaw cycles (**do not store in a frost-free freezer**).
- Upon thawing, use what you need and store the thawed stock solution at 4°C for up to 2 weeks.
- Medium containing blasticidin may be stored at 4°C for up to 2 weeks.

Culture Volumes

Optimal Culture Volumes

The table below describes the optimal culture volume for use with a variety of culture vessels. The optimal volume is the volume needed for ideal surface area. The maximum volume is the volume needed for sustained growth.
N/A: not applicable (Kirkpatrick and Shatzman, 1997).
Note: We have found that you can increase the rpm in spinner flasks to prevent clumping.

Vessel	Optimal Volume	Maximum Volume	Optimal RPM	Caps
96-well plate	50 µL/well	200 µL	N/A	Sealed with Parafilm™
24-well plate	300 µL/well	600 µL	N/A	Sealed with Parafilm™
12-well plate	600 µL/well	1 mL	N/A	Sealed with Parafilm™
6-well plate	1.5 mL/well	2.5 mL	N/A	Sealed with Parafilm™
25-cm^2 flask	5 mL	N/A	N/A	Loosened
75-cm^2 flask	15 mL	N/A	N/A	Loosened
150-cm^2 flask	30 mL	N/A	N/A	Loosened
125-mL spinner	70 mL	100 mL	100	Loosened (1/4 turn)
250-mL spinner	120 mL	150 mL	100	Loosened
500-mL spinner	250 mL	300 mL*	80–90	Loosened
1000-mL spinner	400 mL	600 mL	70–80	Loosened
3000-mL spinner	800 mL	1000 mL	70–80	Loosened
250-mL shake flask	100 mL	150 mL	115	Loosened
500-mL shake flask	200 mL	300 mL	115	Loosened
1000-mL shake flask	500 mL	700 mL	115	Loosened
3000-mL shake flask	1000 mL	1200 mL	115	Loosened

*Volume may be increased to 500 mL in Bellco spinner flasks.

Suspension Cell Cultures

Introduction

For large-scale growth and purification, S2 cells can be grown in suspension culture. Use the following protocol as a starting point for scale-up. This protocol can be easily adapted to shake flasks if desired.

Materials Required but not Supplied

- S2 cells in culture (either adherent or in suspension)
- Pluronic® F-68, if desired (see page 21 for ordering information)
- Heparin (Sigma, Cat. no. H3149)
- Complete Schneider's *Drosophila* Medium
- 250-mL spinner flask (other flasks may be used)
- Magnetic stir plate

Before Starting

Optional: You may want to add Pluronic® F-68 to the medium at a concentration of 0.05-0.1%. Pluronic F-68® is a surfactant that prevents the cells from shearing.

Cell Clumping

Cell clumping is more likely to occur in medium containing serum. If cell clumping (>10 cells per clump) occurs, add heparin at 10 Units/mL. Clumping occurs at the higher cell densities (i.e. > 8×10^6 cells/mL in serum containing medium and >30×10^6 cells/mL in serum-free medium).

Procedure

1. For a culture volume of 125–150 mL, use a 250-mL spinner flask. Usually, the culture volume should be about half the total vessel volume (capacity).
2. Inoculate the spinner flask with either serum-free or serum containing medium and seed cells at approximately 1–2 × 10^6 cells/mL and viability of >95%.
3. Incubate the spinner flask at 28°C with a constant stirring rate of 90–125 rpm. Loosen the side arms approximately a quarter (1/4) turn. Increase the rate to 140 rpm when cell densities reach >10 x 10^6 cells/mL.
   - The cell viability begins to decrease at densities >12 × 10^6 cells/mL in medium-containing serum. Keep cell densities between 5–10 × 10^6 cells/mL in serum-containing medium.
   - Cell densities can approach 30 × 10^6 cells/mL without significant decrease in viability in serum-free medium.
4. Subculture cells to ~5 × 10^6 cells/mL when densities reach about ~10 × 10^6 cells/mL.

   Note: If cells are subcultured at densities below 1 × 10^6 cells/mL, the growth rate will significantly decrease.

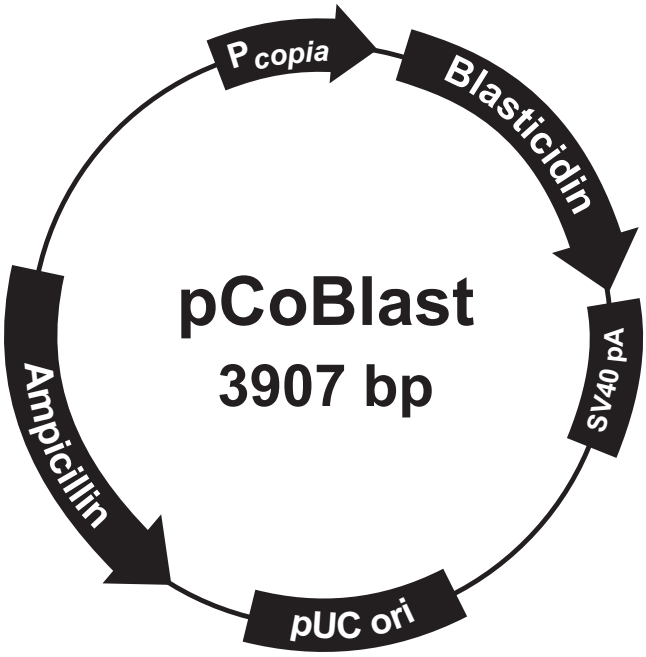
pCoBlast Map and Features

Description

pCoBlast is a 3907 bp selection vector that can be cotransfected with the expression vector of your choice to create stable cell lines in S2 cells. It contains the *Streptomyces griseochromogenes bsd* gene (Kimura *et al.*, 1994) under control of the *Drosophila copia* promoter.

Map

The figure below summarizes the features of the pCoBlast vector. **The nucleotide sequence for pCoBlast is available from www.lifetechnologies.com or by contacting Technical Support (see page 22).**



Comments for pCoBlast 3907 nucleotides

copia promoter: bases 500-746
Blasticidin resistance gene: bases 797-1192
SV40 early polyadenylation sequence: bases 1527-1661
pUC origin: bases 2029-2702 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 2847-3707 (complementary strand)
bla promoter: bases 3708-3806 (complementary strand)

Continued on next page

pCoBlast Map and Features, Continued

Features of pCoBlast

The table below describes the relevant features of pCoBlast. All features have been functionally tested.

Feature	Benefit
Drosophila copia promoter	Allows high-level expression of the blasticidin resistance gene.
Blasticidin (*bsd*) resistance gene	Allows selection of stable transfectants in *Drosophila* S2 cells (Kimura *et al.*, 1994).
SV40 early polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA.
pUC origin	Allows high-copy number replication and growth in *E. coli.*
bla promoter	Allows expression of the ampicillin (*bla*) resistance gene.
Ampicillin (*bla*) resistance gene (β-lactamase)	Allows selection of transformants in *E. coli.*

Accessory Products

Introduction

The products listed in this section are intended for use with the DES® Blasticidin Support Kit. For more information, visit **www.lifetechnologies.com** or call Technical Support (see page 22).

Additional Products

Many of the reagents supplied in the DES® Blasticidin Support Kit as well as additional products that may be used with the DES® Blasticidin Support Kit are available separately. Ordering information is provided below.

Product	Amount	Cat. no.
Drosophila S2 Cells	1 mL vial, 1 x 10^7 cells/mL	R690-07
Schneider's *Drosophila* Medium	500 mL	21720-024
Certified Fetal Bovine Serum, US	500 mL	16000-044
Penicillin-Streptomycin	100 mL	15070-063
Calcium Phosphate Transfection Kit	75 reactions	K2780-01
Blasticidin S HCl	50 mg	R210-01
pMT/V5-His A, B, and C	20 µg each	V4120-20
pMT/BiP/V5-His A, B, and C	20 µg each	V4130-20
pAc5.1/V5-His A, B, and C	20 µg each	V4110-20
DES® TOPO® TA Expression Kit	1 kit (20 reactions)	K4125-01
Phosphate Buffered Saline, pH 7.4	500 mL	10010-023
One Shot® TOP10 (chemically competent cells)	21 × 50 µL	C4040-03
One Shot® TOP10 Electrocomp™ (electrocompetent cells)	21 × 50 µL	C4040-52
One Shot® DH5α™-T1^R Max Efficiency® (chemically competent cells)	21 × 50 µL	12297-016
imMedia™ Amp Agar	20 each	Q60120
PureLink® HiPure Filter Plasmid Kit	25 preps	K2100-14
Pluronic® F-68, 10% (100X)	100 mL	24040-032

DES® Kits

The DES® Blasticidin Support Kit is designed for use in conjunction with a number of DES® System Kits available from Life Technologies. For more information about the DES® System Kits, visit **www.lifetechnologies.com** or call Technical Support (see page 22). Ordering information is provided below.

Product	Amount	Cat. no.
DES® Inducible Kit with pCoBlast	1 kit	K5120-01
DES® Inducible/Secreted Kit with pCoBlast	1 kit	K5130-01
DES® BioEase™ Expression Kit with pCoBlast	20 reactions	K4140-01

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com/support**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Use Label License No. 51: Blasticidin and the Blasticidin Selection Marker

Blasticidin and the blasticidin resistance gene (*bsd*) are the subject of a U.S. Patent sold under patent license for research purposes only. Inquiries for commercial use should be directed to: Kaken Pharmaceutical Company, Ltd., Bunkyo Green Court, Center Office Building, 19-20 Fl, 28-8 Honkomagome 2-chome, Bunkyo-ku, Tokyo 113-8650, Japan, Tel: 81 3-5977-5008; Fax: 81 3-5977-5008.

Limited Use Label License No. 56: Drosophila Expression System (DES®)

This product is licensed to you under patent license from SmithKline Beecham Corporation for your research use only. This product may not be sold or transferred to any other person. In using this product, you agree to make no commercial use of this product. Requests for licenses for commercial manufacture or use may be made directly to **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Please note that you need a license to practice this technology. If you do not have a signed agreement on file, please use the contact information above to obtain a license agreement.

References

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current Protocols in Molecular Biology (New York: Greene Publishing Associates and Wiley-Interscience).

Bunch, T. A., Grinblat, Y., and Goldstein, L. S. (1988). Characterization and Use of the *Drosophila* Metallothionein Promoter in Cultured *Drosophila melanogaster* Cells. Nucleic Acids Res. *16*, 1043-1061.

Chung, Y. T., and Keller, E. B. (1990). Positive and Negative Regulatory Elements Mediating Transcription from the *Drosophila melanogaster* Actin 5C Distal Promoter. Mol. Cell. Biol. *10*, 6172-6180.

Coligan, J. E., Dunn, B. M., Ploegh, H. L., Speicher, D. W., and Wingfield, P. T. (1998). Current Protocols in Protein Science (New York: John Wiley).

Deutscher, M. P. (1990) Guide to Protein Purification. In *Methods in Enzymology*, Vol. 182. (J. N. Abelson and M. I. Simon, eds.) Academic Press, San Diego, CA.

Han, K. (1996). An Efficient DDAB-Mediated Transfection of *Drosophila* S2 Cells. Nucleic Acids Res. *24*, 4362-4363.

Izumi, M., Miyazawa, H., Kamakura, T., Yamaguchi, I., Endo, T., and Hanaoka, F. (1991). Blasticidin S-Resistance Gene (*bsr*): A Novel Selectable Marker for Mammalian Cells. Exp. Cell Res. *197*, 229-233.

Kimura, M., Takatsuki, A., and Yamaguchi, I. (1994). Blasticidin S Deaminase Gene from *Aspergillus terreus* (*BSD*): A New Drug Resistance Gene for Transfection of Mammalian Cells. Biochim. Biophys. Acta *1219*, 653-659.

Kirkpatrick, R., and Shatzman, A. (1997). Personal communication.

Maroni, G., Otto, E., and Lastowski-Perry, D. (1986). Molecular and Cytogenetic Characterization of a Metallothionein Gene of *Drosophila*. Genetics *112*, 493-504.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Plainview, New York: Cold Spring Harbor Laboratory Press).

Schneider, I. (1972). Cell Lines Derived from Late Embryonic Stages of *Drosophila melanogaster*. J. Embryol. Exp. Morph. *27*, 363-365.

Takeuchi, S., Hirayama, K., Ueda, K., Sakai, H., and Yonehara, H. (1958). Blasticidin S, A New Antibiotic. The Journal of Antibiotics, Series A *11*, 1-5.

Yamaguchi, H., Yamamoto, C., and Tanaka, N. (1965). Inhibition of Protein Synthesis by Blasticidin S. I. Studies with Cell-free Systems from Bacterial and Mammalian Cells. J. Biochem. (Tokyo) *57*, 667-677.

©2012 Life Technologies Corporation. All rights reserved.

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Notes

Notes

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.invitrogen.com/support or email techsupport@invitrogen.com

www.lifetechnologies.com

life technologies™